



Instructions for use (English)

1 Purpose

Only for research purposes!

2 Field of application

The recomLine SARS-CoV-2 IgG [Avidität] is a line immunoassay. The separate line-up of individual antigens means that, unlike ELISA, the test allows identification of specific antibodies against the individual antigens of the various coronaviruses. The test uses for SARS-CoV-2 (severe acute respiratory syndrome Coronavirus 2) the following recombinant antigens: NP (nucleocapsid), RBD (receptor binding domain of the spike protein) and S1 (S1 subunit of the spike protein). For the seasonally occurring human coronaviruses (HCoV: NL63, OC43, 229E, HKU1) nucleocapsid proteins (NP) are used. For the avidity determination of the IgG antibodies, the avidity reagent can be requested separately.

This test must not be used for clinical diagnosis!

3 Test principle

Highly purified recombinant antigens (NP, RBD and S1 for SARS-CoV-2, as well as NP for NL63, OC43, 229E, HKU1) are fixed to nitrocellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma sample with specific antibodies binding to the pathogen antigens on the test strips.
2. Unbound antibodies are then washed away.
3. The strips are incubated in a second step with anti-human immunoglobulin antibodies (IgG) that are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then washed away.
5. Specific bound antibody is detected in a colour reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band appears on the strip at the corresponding location.

Control bands are located at the top end of the test strip:

- a) The reaction control below the strip number for which every serum/plasma sample must show a reaction.
- b) The conjugate controls (IgG) are used to check the conjugate and strip type used (Ig class specific).
- c) 'Cut-off control': The intensity of this band enables an evaluation of the reactivity of the individual antigen bands (see 9.2 Analysis).

4 Reagents

4.1 Package contents

The reagents in one pack are sufficient for 20 assays. Each set of reagents contains:

WASHBUF A 10 X	100 ml wash buffer A (10x concentrate) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxyprion (0.2%)
SUBS TMB	40 ml chromogenic substrate tetramethylbenzidine (TMB, ready-to-use)
MILKPOW	5 g low-fat milk powder
INSTRU	1 instructions for use
EVALFORM	1 evaluation sheet

4.1.1 recomLine SARS-CoV-2 IgG [Avidität]

In addition to the components listed under section 4.1, each set of reagents contains:

TESTSTR	2 tubes each with 10 consecutively numbered test strips
CONJ IgG	500 µl anti-human IgG conjugate (100x concentrate, green cap) From rabbit, contains NaN ₃ (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)

4.1.2 Avidity determination

To determine the avidity of coronavirus IgG antibodies, the avidity reagent with the corresponding instructions for use can be supplied upon request as a supplement.

AVIDI	1 avidity reagent (solid 25 g) for 60 ml ready-to-use solution
Article no. 11010	

4.2 Additionally required reagents, materials and equipment

- Incubation trays (can be purchased from MIKROGEN as required)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotatory device
- Vacuum pump or similar device
- Measuring cylinder 50 ml and 1000 ml
- Micropipettes with single-use tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for biohazardous substances

5 Shelf life and handling

- ☞ Store reagents between +2 °C and +8 °C before and after use; **do not freeze.**
- ☞ Before starting the test, allow all reagents sit at room temperature (+18 °C to +25 °C) for at least 30 minutes. The test is carried out at room temperature.
- ☞ Wash buffer, milk powder, dilution buffer, conjugates and TMB can be exchanged between the different recomLine and/or recomBlot test systems if these components carry the same symbol. Note the shelf life of these components when doing so.
- ☞ Before use, mix the concentrated reagents and patient sera thoroughly. Avoid foam formation.
- ☞ Only open the tubes with the test strips just before use to prevent water condensation. Strips that are not needed must be left in the tube and continue to be stored at +2 °C to +8 °C (reseal tube firmly, test strips must not be moist before the start of the test!).
- ☞ The strips are identified with a consecutive number and a test abbreviation.
- ☞ The packages have an expiry date, after which no further guarantee of quality can be given.
- ☞ Keep the kit components away from direct sunlight throughout the test procedure. Note: The substrate solution (TMB) is light sensitive.
- ☞ The test must only be performed by trained, authorised and qualified personnel.
- ☞ Substantial changes made by the user to the product or the directions for use may compromise the intended purpose of the test specified by Mikrogen.
- ☞ Cross-contamination of the patient samples or conjugates in the kit can lead to false test results. Add patient samples, test strips and conjugate solution carefully. Make sure that any incubated liquids are not carried over to other wells. Carefully remove liquids.
- ☞ The strips must be completely wetted and submersed throughout the entire procedure.
- ☞ Automation is possible. Further details are available from Mikrogen.

6 Warnings and safety precautions

- ☞ Only for research purposes.
- ☞ All blood products must be treated as potentially infectious.
- ☞ The test strips were manufactured with inactivated whole-cell lysates and/or recombinant bacterial, viral or parasitic antigens.
- ☞ After adding patient or control material, the strips must be considered to be potentially infectious and handled appropriately as such.
- ☞ Suitable single-use gloves must be worn throughout the entire test procedure.
- ☞ The reagents contain the antimicrobial agents and preservatives sodium azide (NaN₃), MIT (methylisothiazolinone), Oxyprion and chloroacetamide. Avoid contact with the skin or mucous

membranes. Sodium azide (NaN₃) can form explosive azides if it comes into contact with heavy metals such as copper and lead.

- ♻️ All aspirated liquids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens or be autoclaved. All reagents and materials that come into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentrations and incubation times specified by the manufacturer must be followed.
- ♻️ Only use incubation trays once.
- ♻️ Handle strips carefully with a pair of plastic forceps.
- ♻️ Do not replace or mix the reagents with reagents of other manufacturers.
- ♻️ Read through and carefully follow all instructions before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and preparation

7.1 Sample material

The sample material can be either serum or plasma (EDTA, citrate, heparin, CPD) and must be separated as soon as possible after collection to avoid haemolysis. Microbial contamination of the sample must absolutely be avoided. Non-soluble substances must be removed from the sample prior to incubation. Use of icteric, haemolytic, lipaemic or cloudy samples is not recommended.

Caution!

If the tests are not carried out immediately, the sample material can be stored for up to 2 weeks between +2 °C and +8 °C. It is possible to store the samples for longer periods at -20 °C or lower. Repeated freezing and thawing of the samples is not recommended due to the risk of false results. More than 3 freezing and thawing cycles should be avoided.

7.2 Preparation of solutions

7.2.1 Preparation of the ready-to-use wash buffer A

This buffer is required for the serum and conjugate dilution and the wash steps.

Before the dilution, the volume of wash buffer A must be determined for the corresponding number of tests to be carried out.

The low-fat milk powder is first pre-dissolved in wash buffer A concentrate and then this mixture is made up to the final volume with deionised water (dilution 1 + 9). The required quantity for a defined number of test strips is calculated using the following formulae (device-specific dead volumes are not taken into account):

Reagent	Formula	Example: 5 strips
Low-fat milk powder [g]	= strip number × 0.1	0.5 g
Wash buffer A concentrate [ml]	= strip number × 2	10 ml
Deionised water [ml]	= strip number × 18	90 ml
Ready-to-use wash buffer A [ml]	= strip number × 20	100 ml

Ready-to-use wash buffer A can be stored at +2 °C to +8 °C for four weeks. The ready-to-use wash buffer A has no odour and is slightly turbid.

7.2.2 Preparation of conjugate solutions

The conjugate solution must be prepared shortly before use; the ready-to-use conjugate solution must not be stored.

One part of the conjugate concentrate is diluted with 100 parts of the ready-to-use wash buffer A (1 + 100).

The required quantity for a defined number of test strips is calculated using the following formulae:

Reagent	Formula	Example: 5 strips
Conjugate concentration [µl]	= strip number × 20	100 µl
Ready-to-use wash buffer A [ml]	= strip number × 2	10 ml

The conjugate quantities are calculated without dead volumes. Depending on the processing (manually or with a device), please prepare additional conjugate solution for 1 to 3 strips.

8 Test procedure

No.	Implementation	Note
1	Before starting the test, allow all reagents to sit between 18 °C and 25 °C (room temperature) for at least 30 minutes.	The test is carried out at room temperature.
2	Prepare the test strips Place strips in 2 ml ready-to-use wash buffer A (see 7.2.1).	Do not handle the strips with bare hands – use forceps. The strip number faces upwards. For each strip one well in an incubation tray (see 4.1.1) is required. The strips must be completed submersed.
3	Sample incubation a) 20 µl of an undiluted sample (human serum or plasma) are added by pipette to the test strip for each incubation mixture (dilution 1 + 100). b) Incubate for 1 hour with gentle shaking.	Pipette the sample onto one end of the submersed strip in wash buffer A and mix as quickly as possible by gently shaking the incubation bath. Cover the incubation tray with the plastic lid and place on the shaker.
4	Wash a) Carefully remove the plastic lid from the incubation tray. b) Carefully aspirate the serum dilution from the individual wells. c) Pipette 2 ml ready-to-use wash buffer A (see 7.2.1) into each well, wash for 5 minutes with gentle shaking and then aspirate off the wash buffer A.	Carry out wash steps 8.4a–8.4c a total of three times. Avoid cross-contamination. With automated processing, follow the directions of the device manufacturer for this step.
5	Incubation with conjugate Add 2 ml ready-to-use conjugate solution (see 7.2.2) and incubate for 45 minutes with gentle shaking.	Cover the incubation tray with the plastic lid and place on the shaker.
6	Wash See section 8.4	Carry out wash steps a total of three times (see 8.4a–8.4c).
7	Substrate reaction Add 1.5 ml substrate solution and incubate for 8 minutes with gentle shaking.	
8	Stopping the reaction Remove the substrate solution. Wash at least three times briefly with deionised water .	
9	Drying the strips Dry the strips before the analysis for 2 hours between 2 layers of absorbent paper.	Carefully remove the strips from the water with a pair of plastic forceps. Store the strips protected from light.
Caution! Incubation solutions must not be carried over to other wells. Avoid splashing, particularly when opening and closing the cover.		

9 Results

Caution:

Do not use the automated interpretation without noting the information described below about the interpretation.

9.1 Validation – Quality control

The test can only be analysed when the following criteria are satisfied:

1. Reaction control band (upper line) is clearly stained, dark band detectable.
2. Antibody class (second band): the IgG conjugate control band must be clearly stained.
3. Cut-off control (third band): weak but visible staining.

9.2 Analysis

The test strips can be analysed visually or with a computer using the *recomScan* test strip analysis software. The *recomScan* software is intended to help with test strip interpretation. Additional information and corresponding instructions for computer-aided analysis are available from MIKROGEN upon request. The following instruction refers to the visual analysis.

9.2.1 Evaluation of the band intensity

- Note the date and batch number along with the antibody class that was detected in the attached evaluation form.
- Enter the sample identification numbers in the evaluation form.
- Now adhere the associated test strip with a glue stick into the corresponding field in the evaluation form. Align the test strip with the reaction control band at the indicated marker line. Then adhere the test strip to the left of the marking line using transparent adhesive tape (do not stick over the reaction control band!). Adhering the entire test strip with glue stick or adhesive tape can lead to changes in the staining.
- Now identify the bands for the developed test strips using the printed control strip from the evaluation form and enter these into the evaluation form. Carry out the evaluation of the intensity of the emergent bands separately for the relevant immunoglobulin classes using Table 1.

Table 1: Evaluation of the band intensity relative to the cut-off band

Colour intensity of the bands	Evaluation
No reaction	-
Very weak intensity (less than the cut-off band)	+/-
Weak intensity (corresponds to the cut-off band)	+
High intensity (greater than the cut-off band)	++
Very high intensity	+++

Evaluation of the avidity: see section 9.4.

9.3 Interpretation of test results

Test interpretation of recomLine SARS-CoV-2 IgG [Avidität]

Test interpretation	Antigen reactivity
SARS-CoV-2 IgG positive	One or more SARS-CoV-2 specific antigen bands (NP, RBD und/oder S1) are positive (irrespective of the reactivity of the HCoV antigens)
SARS-CoV-2 IgG negative	All SARS-CoV-2 specific antigen bands (NP, RBD und/oder S1) are negative (irrespective of the reactivity of the HCoV antigens)

9.4 Addition of the avidity determination

IgG antibodies mature with antibodies in the earlier stages having low avidity while antibodies have higher avidity once an infection has passed. Low avidity antibodies can be washed off the binding site on the strip using an avidity reagent while high avidity antibodies are not able to be detached by the reagent. By processing two IgG strips in parallel, of which one has to be treated with the avidity reagent, it can be determined whether the IgG antibodies have low avidity (acute infection) or high avidity (past infection).

9.4.1 Test principle and test procedure

The avidity of coronavirus IgG antibodies can be determined using the avidity reagent, article no. 11010. The test procedure can be found in the instructions for use for the avidity reagent.

9.4.2 Analysis of the avidity in the recomLine SARS-CoV-2 IgG [Avidität]

- Only carry out the avidity determination with an overall positive IgG result.
- Bands on the IgG strip that have a lower reactivity than the cut-off are not considered in the avidity determination.
- Compare the intensities of the corresponding bands on the two test strips (IgG strip and avidity strip) that were incubated with the same patient sample. Note whether the intensities have changed.
- A reduction in the intensity of the bands by more than 40% can be considered as low avidity.
- With high avidity, the band intensity of the avidity strip reduces by 40% or less and the IgG antibodies are considered to have high avidity.
- In general, there are no absolute rules that apply for the avidity evaluation. In some cases it must be noted that low avidity cannot be ruled out even for previous infections because there may have been a delay in the maturation of the avidity.

10 Literature

- S. Kannan, P. Shaik Syed Ali, A. Sheeza, K. Hemalatha. COVID-19 (Novel Coronavirus 2019) – recent trends. Eu. Rev. Med. Pharmacol. Sci. 2020;24:2006-2011
- F. Amanat, T. H.O. Nguyen, V. Chromikova, S. Strohmeier, D. Stadlbauer, A. Javier, K. Jiang, G. A. Arunkumar, J. Polanco, M. Bermudez-Gonzales, D. Caplivski, A. Cheng, K. Kedzierska, O. Vapalahti, J. M. Hepojoki, V. Simon, F. Krammer. A serological assay to detect SARS-CoV-2 seroconversion in humans. Preprint

- N Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. Emerg Infect Dis. 2020 Jul

We will be pleased to send you additional literature on request.

11 Explanation of symbols

	Content is sufficient for <n> formulations Number of formulations
WASHBUF A 10 X	Wash buffer A (10x concentrate)
SUBS TMB	Chromogenic substrate tetramethylbenzidine
MILKPOW	Low-fat milk powder
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
AVIDI	Avidity reagent
ADD	Additional reagent, available on request
EVALLFORM	Evaluation sheet
INSTRU	Instructions for use
	Follow the instructions for use
CONT	Contents, contains
RUO	Research Use Only
LOT	Batch number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store between x °C and y °C
	Manufacturer

12 Manufacturer and version data

recomLine SARS-CoV-2 IgG [Avidität]	Article no. 7374
Instructions for use	GARLCS001RUOEN
Valid from	2020-06
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GARLCS001RUO